Clinical Trial Protocol

	Document Number:	**c25939630-02**
EudraCT No.	2018-003745-41	
BI Trial No.	1386-0022	
BI Investigational Medicinal Product	BI 1467335	
Title	A Phase I, open-label, Positron Emission Tomography study in healthy male subjects to explore the inhibition of monoamine oxidase B in the brain after multiple oral doses of BI 1467335 (non-randomized, open-label, parallel-group study)	
Lay Title	A study in healthy men to test the effects of different doses of BI 1467335 on MAO-B activity in the brain.	
Clinical Phase	I	
Clinical Trial Leader	Phone: Fax:	
Principal Investigator	Phone:	
Status	Final Protocol (Revised Protocol (based on global amendment 1))	
Version and Date	Version: 2.0	Date: 24 Jun 2019

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	07 January 2019
Revision date	24 June 2019
BI trial number	1386-0022
Title of trial	A Phase I, open-label, Positron Emission Tomography study in healthy male subjects to explore the inhibition of monoamine oxidase B in the brain after multiple oral doses of BI 1467335 (non-randomized, open-label, parallel-group study).
Principal Investigator:	
Trial sites	
Clinical phase	I
Trial rationale	*In vitro* data suggests an irreversible MAO-B inhibition in humans by BI 1467335. This trial is intended to identify and quantify MAO-B inhibition in the brain following multiple oral administration of BI 1467335 in healthy male subjects to exclude a potential clinical relevance.
Trial objective	To investigate the effect of multiple oral dosing of 10 mg BI 1467335 over 28 days and 3 mg BI 1467335 over 42 days compared to baseline on MAO-B inhibition in the brain using [^{11}C]-L-deprenyl-D2 PET tracer.
Trial design	Non-randomised, open-label, parallel-group, multiple-dose design.
Trial endpoints:	Primary endpoint: - % reduction in MAO-B availability upon treatment with BI 1467335 on the last treatment day (Day 28 for the 10 mg dose group and Day 42 for the 3 mg dose group) compared to baseline. Secondary endpoints: - % reduction in MAO-B availability upon treatment with 10 mg BI 1467335 on Day 14 compared to baseline. - % reduction in MAO-B availability upon treatment with 3 mg BI 1467335 on Day 28 compared to baseline. - MAO-B inhibition in platelet rich plasma at Day 14 (10 mg dose group only), Day 28, and Day 42 (3 mg dose group only) compared to baseline. - $C_{max,N}$, and $AUC_{0-24,N}$ on Day 14 (10 mg dose group only), Day 28 and Day 42 (3 mg dose group only).

Number of subjects	
total entered	10
each treatment	5
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male subjects, age of 21 to 55 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive), non-smoker
Test products	BI 1467335 film-coated tablet formulation (dose strengths 5 mg and 1 mg)
dose	Dose group 1: 10 mg of free base (2 x 5 mg) Dose group 2: 3 mg of free base (3 x 1 mg)
mode of admin.	Oral with 240 mL of water
Diagnostic tracer	[^{11}C]-L-deprenyl-D2
dose	≤ 100 µg
mode of admin.	Intravenous bolus injection
Duration of treatment	10 mg BI 1467335: Once daily for twenty-eight days. 3 mg BI 1467335: Once daily for forty-two days. [^{11}C]-L-deprenyl-D2: One single intravenous bolus injection before each PET scan, between Day -14 to Day -2 for a predose assessment, on Day 28 for both dose groups and additionally on Day 14 for the 10 mg dose group only and on Day 42 for the 3 mg dose group only.
Statistical methods	will conduct image analysis to generate the outcome parameter for the PET data (proportional to the target availability at each PET scan), using the PET emission and the metabolite corrected arterial plasma input function, within an appropriate kinetic model. Tissue time-activity curves (TACs) for each region of interest (ROI) will be analysed together with arterial blood data, processed to generate metabolite-corrected arterial plasma input function curves. Kinetic modelling techniques will then be applied to the input function and TAC data to estimate parameters relating to the level of target availability for each PET scan. For scans following 14 or 28 days of dosing with 10 mg BI 1467335 and 28 or 42 days of dosing with 3 mg BI 1467335, fractional occupancy values will be generated for each post-dose PET scan by comparing the outcome parameters for baseline and post-dose scans for a subject (primary and secondary MAO-B inhibition endpoints). The occupancy data will be related to the plasma concentration of BI 1467335 at the time of the PET scan. Descriptive statistics will be calculated for all endpoints.

FLOW CHART

DOSE GROUP 3 mg BI 1467335

Visit	Day	Planned time (relative to first oral drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK_{plasma}[15]	PET procedure[8]	Platelet-enriched plasma	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[10]
1	-28 to -2			Screening (SCR)[1]	x				x	x	
	-14 to -2			Predose PET scan[13]			x				
2	-1	-14:00	18:00	Admission to clinical trial site[9]	x[5]						
	1	-2:00	07:00		x[2, 4]	x[2]		x	x[2]	x[2]	
		0:00	09:00	Drug administration							
	2 to 6	24:00 to 120:00	09:00	Drug administration							
	7	144:00	09:00	Drug administration	x[5]				x[2]	x[2]	
	8 to 12	168:00 to 264:00	09:00	Drug administration							
	13	288:00	09:00	Drug administration							
	14	312:00	09:00	Drug administration	x[2, 4, 5]				x[2]	x[2]	
	15 to 20	336:00 to 456:00	09:00	Drug administration							
	21	480:00	09:00	Drug administration					x[2]	x[2]	
	22 to 27	504:00 to 624:00	09:00	Drug administration							
	24	552:00	09:00	Drug administration							
	25	576:00	09:00	Drug administration							
	26	600:00	09:00	Drug administration							
	27	624:00	09:00	Drug administration							
	28	648:00	09:00	Drug administration	x[2, 4, 11]	x[2]			x[2]	x[2]	
		648:20	09:20			x					
		648:45	09:45			x					
		649:00	10:00			x					
		649:30	10:30			x	x	x			
		651:00	12:00			x					
		652:00	13:00	Lunch, 240 mL water intake[3]		x					
		655:00	16:00	Snack[3]		x					
		657:00	18:00	Dinner[3]					x	x	
		659:00	20:00			x					
	29	672:00	09:00	Drug administration		x[2]					
	30	696:00	09:00	Drug administration							
	31	720:00	09:00	Drug administration							
	32	744:00	09:00	Drug administration							
	33	768:00	09:00	Drug administration							

Visit	Day	Planned time (relative to first oral drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK_{plasma}[15]	PET procedure[8]	Platelet-enriched plasma	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[10]
2	34	792:00	09:00	Drug administration							
	35	816:00	09:00	Drug administration					x[2]	x[2]	
	36	840:00	09:00	Drug administration							
	37	864:00	09:00	Drug administration							
	38	888:00	09:00	Drug administration							
	39	912:00	09:00	Drug administration							
	40	936:00	09:00	Drug administration							
	41	960:00	09:00	Drug administration					x[2]	x[2]	
	42	984:00	09:00	Drug administration	x[2, 4, 11]	x[2]			x[2]	x[2]	
		984:20	09:20			x					
		984:45	09:45			x					
		985:00	10:00			x					
		985:30	10:30			x	x	x			
		987:00	12:00			x					
		988:00	13:00	Lunch, 240 mL water intake[3]		x					
		991:00	16:00	Snack[3]		x					
		993:00	18:00	Dinner[3]					x	x	
		995:00	20:00			x					
	43	1008:00	09:00	Discharge from trial site and confirmation of fitness[12]		x			x	x	
3	47-50			End of trial (EoTrial) examination[6, 7]	x				x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug, alcohol, nicotine and virus screening), demographics (including determination of body height and weight, BMI, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria. In case subject is considered eligible for study participation, an MRI will be conducted as part of the screening procedures.
2. The time is approximate; the procedure is to be performed and completed within a time window of 3 h prior to drug administration. After start of treatment on Day 1, further predose PK blood sampling should be performed within 30 minutes before next scheduled dosing.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.
4. Subjects are to be fasted for at least 10 h before sample is taken.
5. Only urine drug, alcohol screening and assessment of urine cotinine to confirm no smoking will be done on Day -1. On Day 7 and Day 14, these assessments will only be performed upon re-admission, in case the subject leaves the unit for the afternoon.
6. End-of-trial (EoTrial) examination to be performed on Day 47-50, EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
7. For definition of the individual subject's end of trial see Section 6.2.3.
8. Dynamic PET scan will be acquired from 0 to 90 minutes after injection of the PET ligand [^{11}C]-L-deprenyl-D2. In parallel arterial blood samples will be collected continuously for 15 min using an automated blood sampling system at a speed of 5 mL/min. In addition, arterial blood will be drawn manually at several time points during the PET scan.

9. Subjects will be hospitalized at the clinical trial site from admission on Day -1 to Day 43. On Day 7 and Day 14, subjects will be allowed to leave the unit in the afternoon.
10. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
11. On trial days 28 and 42 subjects will be allowed a light breakfast after safety assessments have been completed.
12. Discharge from trial site after completion of all assessments, including physical examination for confirmation of fitness.
13. Predose PET scan will only take place once subjects have completed all screening procedures (including MRI) and they are considered eligible for study participation.

15. On the days of the PET scans, any PK samples that would need to be taken during the PET scan, can be taken earlier or later, if required. Actual sampling times will be recorded in the CRF.

DOSE GROUP 10 mg BI 1467335

Visit	Day	Planned time (relative to first oral drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK_{plasma}[15]	PET procedure[8]	Platelet-enriched plasma	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[10]
1	-28 to -2			Screening (SCR)[1]	x				x	x	
	-14 to -2			Predose PET scan[13]			x				
2	-1	-14:00	18:00	Admission to clinical trial site[9]	x[5]						↕
	1	-2:00	07:00		x[2, 4,]	x[2]		x	x[2]	x[2]	
		0:00	09:00	Drug administration							
	2	24:00	09:00	Drug administration							
	3	48:00	09:00	Drug administration							
	4	72:00	09:00	Drug administration					x[2]	x[2]	
	5	96:00	09:00	Drug administration							
	6	120:00	09:00	Drug administration							
	7	144:00	09:00	Drug administration					x[2]	x[2]	
	8	168:00	09:00	Drug administration							
	9	192:00	09:00	Drug administration							
	10	216:00	09:00	Drug administration							
	11	240:00	09:00	Drug administration							
	12	264:00	09:00	Drug administration							
	13	288:00	09:00	Drug administration							
	14	312:00	09:00	Drug administration	x[2, 4]	x[2]			x[2]	x[2]	
		312:20	09:20			x					
		312:45	09:45			x					
		313:00	10:00			x					
		313:30	10:30			x	x	x			
		315:00	12:00			x					
		316:00	13:00	Lunch, 240 mL water intake[3]		x					
		319:00	16:00	Snack[3]		x					
		321:00	18:00	Dinner[3]					x	x	
		323:00	20:00			x					
	15	336:00	09:00	Drug administration		x[2]					
	16	360:00	09:00	Drug administration							
	17	384:00	09:00	Drug administration							
	18	408:00	09:00	Drug administration							
	19	432:00	09:00	Drug administration							
	20	456:00	09:00	Drug administration							
	21	480:00	09:00	Drug administration					x[2]	x[2]	
	22	504:00	09:00	Drug administration							

Visit	Day	Planned time (relative to first oral drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK_{plasma}	PET procedure[8]	Platelet-enriched plasma	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[10]
2	23	528:00	09:00	Drug administration							↕
	24	552:00	09:00	Drug administration							
	25	576:00	09:00	Drug administration							
	26	600:00	09:00	Drug administration							
	27	624:00	09:00	Drug administration							
	28	648:00	09:00	Drug administration	$x^{2,4}$	x^2			x^2	x^2	
		648:20	09:20			x					
		648:45	09:45			x					
		649:00	10:00			x					
		649:30	10:30			x	x	x			
		651:00	12:00			x					
		652:00	13:00	Lunch, 240 mL water intake[3]		x					
		655:00	16:00	Snack[3]		x					
		657:00	18:00	Dinner[3]					x	x	
		659:00	20:00			x					
	29	672:00	09:00	Discharge from trial site and confirmation of fitness[12]		x			x	x	
3	33-36			End of trial (EoTrial) examination[6, 7]	x				x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug, alcohol, nicotine and virus screening), demographics (including determination of body height and weight, BMI, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria. In case subject is considered eligible for study participation, an MRI will be conducted as part of the screening procedures.
2. The time is approximate; the procedure is to be performed and completed within a time window of 3 h prior to drug administration. After start of treatment on Day 1, further predose PK blood sampling should be performed within 30 minutes before next scheduled dosing.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.
4. Subjects are to be fasted for at least 10 h before sample is taken.
5. Only urine drug, alcohol screening and assessment of urine cotinine to confirm no smoking will be done at this time point.
6. End-of-trial (EoTrial) examination to be performed on Day 33-36, EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
7. For definition of the individual subject's end of trial see Section 6.2.3.
8. Dynamic PET scan will be acquired from 0 to 90 minutes after injection of the PET ligand [^{11}C]-L-deprenyl-D2. In parallel arterial blood samples will be collected continuously for 15 min using an automated blood sampling system at a speed of 5 mL/min. In addition, arterial blood will be drawn manually at several time points during the PET scan
9. Subjects will be hospitalized from admission on Day -1 up to Day 29.
10. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
11. On trial days 14 and 28, subjects will be allowed a light breakfast after safety assessments have been completed.

12. Discharge from trial site after completion of all assessments, including physical examination for confirmation of fitness.
13. Predose PET scan will only take place once subjects have completed all screening procedures (including MRI) and they are considered eligible for study participation.

15. On the days of the PET scans, any PK samples that would need to be taken during the PET scan, can be taken earlier or later, if required. Actual sampling times will be recorded in the CRF.

TABLE OF CONTENTS

ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
AE	Adverse event
AESI	Adverse events of special interest
ALCOA	Attributable, legible, contemporaneous, original, accurate
ALT	Alanine amino transferase
AOC3	Amine oxidase copper-containing 3
AST	Aspartate amino transferase
AUC_{0-24}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the time 24 h
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BMS	Biomarker treated set (TS)
BP	Blood pressure
CA	Competent authority
C_{max}	Maximum measured concentration of the analyte in plasma
$C_{max,N}$	maximum measured concentration of the analyte
CRF	Case Report Form, paper or electronic (sometimes referred to as ‘eCRF’)
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTM	Clinical Trial Manager
CTP	Clinical trial protocol
CTR	Clinical trial report
DILI	Drug induced liver injury
DNA	Deoxyribonucleic acid
EC	Ethics committee
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture

EDTA	Ethylenediaminetetraacetic acid
EoTrial	End of trial
EU	European Union
EudraCT	European Clinical Trials Database
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCL	Hydrochloride
HPLC	High performance liquid chromatography
HR	Heart rate
IB	Investigator's brochure
IC50	Half maximal inhibitory concentration
ICRP	International Commission on Radiological Protection
IEC	Independent Ethics Committee
iPD	Important protocol deviations
IQRMP	integrated risk management plan
IRB	Institutional Review Board
ISF	Investigator site file
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MAO-A/B	Monoamine oxidase A/B
MedDRA	Medical Dictionary for Regulatory Activities
MRD	Multiple-rising dose
MRI	Magnetic Resonance Imaging
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NIMP	Non Investigational Medicinal Product
NOAEL	No observed adverse effect level
PD	Pharmacodynamic(s)
PET	Positron emission tomography
PETS	PET set
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PR	Pulse rate
q.d.	Quaque die, once daily

QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
REP	Residual effect period
ROI	region of interest
SAE	Serious adverse event
SCR	Screening
SOP	Standard operating procedure
SRD	Single-rising dose
SSAO	Semi-carbazide-sensitive amine oxidase
TAC (s)	time-activity curve (s)
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
TS	Treated set
TSAP	Trial statistical analysis plan
UK	United Kingdom
ULN	Upper limit of normal
VAP-1	Vascular adhesion protein-1

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Boehringer Ingelheim (BI) is developing BI 1467335 (formerly Pharmaxis PXS-4728A), an oral, small-molecule inhibitor of semi-carbazide-sensitive amine oxidase (SSAO), also known as vascular adhesion protein-1 (VAP-1) or amine oxidase copper-containing 3 (AOC3), in the indication of non-alcoholic steatohepatitis (NASH).

NASH is characterised histologically by a high level of steatosis, ballooning of hepatocytes, and necroinflammation. NASH often leads to fibrosis which can progress to cirrhosis with a high risk of liver failure. AOC3 in liver sinusoidal endothelial cells is responsible for the firm adhesion and transmigration of leukocytes into the tissue and for the propagation of the inflammatory environment in steatohepatitis. Fibrotic regions of NASH liver sections are strongly positive for AOC3 immune reactivity [R15-5697]. The associated generation of peroxide during the course of amine oxidation is known to activate quiescent stellate cells supporting the differentiation into myofibroblasts, and fibrotic tissue generation. Therefore, targeting the inhibition of AOC3 enzymatic activity might be beneficial for patients with steatohepatitis and fibrosis in order to reduce the recruitment of leukocytes into the liver and reduce cytokine and oxygen stress dependent hepatocyte damage and activation of hepatic stellate cells.

With a prevalence of about 20 - 30% in the general population of Western countries, non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common liver disease worldwide [R15-5365]. While simple hepatic steatosis can have a benign, non-progressive course, about 40% of patients with NAFLD progress to NASH. As the disease progresses, significant fibrosis develops in 37 - 41% of subjects within 15 years. In the United States, NASH is believed to be the most common cause of liver cirrhosis [R15-6070] which is estimated to be the 12th leading cause of death [R15-6057]. Patients with NASH are also at increased risk of hepatocellular carcinoma, even in the absence of cirrhosis [R15-5365]. By 2023, about 13 million patients are projected to have NASH with advanced stages (i.e. ≥ stage 3) of fibrosis (of those, 2.9 million in the US, 3.5 million in EU, 5 million in China). Individuals with advanced fibrosis are estimated to progress with a 4% annual event rate to cirrhosis. The risk of liver-related death in Western patients with NASH ranges from 10% over 13.7 years to 18% over 18.5 years [P13-02280].

To date, no approved therapy for liver fibrosis or effective disease modifying regimen for NASH is available, despite the strong interface with metabolic syndrome, obesity and Type 2 diabetes mellitus. The current standard of care for NASH is weight loss through diet and exercise to improve insulin resistance and lower fat mass which is a clinically challenging goal to achieve and shows minimal impact on disease progression [R15-6044].

For a more detailed description of the BI 1467335 profile please refer to the current Investigator's Brochure (IB) [c04751792].

It should be noted that there are not only ongoing activities to evaluate the potential therapeutic effect of BI 1467335 in patients with NASH [c08980589] but also in patients with non-proliferative diabetic retinopathy [c14141887].

1.2 DRUG PROFILE

1.2.1 BI 1467335

BI 1467335 is a small molecule AOC3 inhibitor that exhibits both anti-inflammatory and anti-fibrotic characteristics in various animal models. AOC3 is a membrane bound adhesion protein that facilitates the binding of leukocytes to endothelial cells and the subsequent transmigration to sites of inflammation.

For a more detailed description of the BI 1467335 profile please refer to the current Investigator's Brochure (IB) [c04751792].

For a more detailed description of the BI 1467335 profile, please refer to the current Investigator's Brochure (IB) [c04751792].

1.2.2 Residual Effect Period

The Residual Effect Period (REP) is the period after the last dose with measurable drug levels and/or pharmacodynamics effects still likely to be present. The REP of BI 1467335 is not known for this clinical trial. Therefore all AEs reported until the end of trial examination (last per protocol contact) will be considered on treatment.

1.2.3 [^{11}C]-L-deprenyl- D2

[^{11}C]-L-deprenyl-D2 is the PET ligand that will be used for the study and is considered a Non Investigational Medicinal Product (NIMP). [^{11}C]-L-deprenyl-D2 is a well-established PET radioligand used over the past 25 years to evaluate MAO-B status in multiple human studies in healthy volunteers and patients with a variety of disorders including epilepsy, traumatic brain injury and Parkinson's disease [R18-2789], [R18-3939], [R18-3940], [R18-3941], [R18-3942], [R18-3943], [R18-3950]. In drug development studies [^{11}C]-L-deprenyl-D2 has been demonstrated to provide reliable information on the occupancy of MAO-B by novel pharmaceutical agents. [^{11}C]-L-deprenyl-D2 has been well tolerated in human studies with no reports of adverse events related to its administration. The total mass of L-deprenyl-D2 administered in this study will not exceed 10 µg at each of the PET scans (as per CHMP guideline of <100 µg (CHMP/ICH/286/95). A qualified physician will be present during radiotracer administration and scanning.

1.3 RATIONALE FOR PERFORMING THE TRIAL

In vitro data for BI 1467335 indicate an irreversible inhibition of MAO-B activity (see Section 1.2). Currently, there is no clinical information regarding brain penetration of BI 1467335 in humans, therefore the clinical relevance of this finding remains open and will be explored with the planned study.

This trial is intended to identify and quantify MAO-B inhibition in the brain in healthy male subjects following multiple oral administration of BI 1467335. MAO-B inhibition will be assessed by PET imaging using [^{11}C]-L-deprenyl-D2 as an established PET ligand. PET imaging will be conducted on predose, Day 14 and Day 28 during a 4-week treatment period with oral doses of once daily 10 mg of BI 1467335 and on predose, Day 28 and Day 42 during a 6-week treatment period with oral doses of once daily 3 mg of BI 1467335. The 10 mg dose group will be investigated first and, in the event no MAO-B inhibition is seen in this dose group, the study will be considered complete and no further investigation with a lower dose (3 mg dose group) will be conducted.

1.4 BENEFIT - RISK ASSESSMENT

Participation in this clinical trial is without any (therapeutic) benefit for healthy subjects. Their participation, however, is of major importance for the development of the compound.

Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication.

Procedure-related risks

The use of an indwelling venous catheter or venepuncture for e.g. blood sampling may result in mild bruising, and in rare cases, in transient inflammation of the wall of the vein, or nerve injury, potentially resulting in paraesthesia, reduced sensibility, and/or pain for an indefinite period.

Subjects will have three PET-CT scans each, with up to 300 MBq [^{11}C]-L-Deprenyl-D2 administered at each of these scans. Each PET-CT scan results in an effective dose of up to 2.0 mSv (PET) + 0.36 mSv (CT) = 2.36 mSv, so the total dose to each subject is up to a maximum of 3 x 2.36 = 7.1 mSv. All of this is over and above standard practice. This is equivalent to 3 times the average yearly exposure (2.3 mSv) from naturally occurring background radiation in the United Kingdom. For an adult in good health, this exposure would result in a risk of around 1 in 2800 of contracting a fatal cancer.

The total exposure falls into category IIb (upper limit 10 mSv) of the guidance published by the International Commission on Radiological Protection (ICRP). There is no dose limit for research purposes in the UK, but like many EU member states, the UK follows the ICRP 62 guidance that recommends 10 mSv (or less) per study where research subjects are not expected to benefit personally.

To mitigate this risk, the study will exclude subjects who have had previous exposure to ionising radiation such that their effective dose resulting from research studies (including this one) would exceed 10 mSv in the previous year.

[^{11}C]-L-Deprenyl-D2 will be injected intravenously as a bolus. The intravenous deprenyl dose will be ≤100 µg, and thus ≥50–100-fold lower than the typical administered dose of 5–10 mg deprenyl to patients with Parkinson's disease [R18-3964]. This low dose of [^{11}C]-L-Deprenyl-D2 is therefore considered to have no noteworthy pharmacological effect in healthy subjects.

The PET investigation includes collection of arterial blood samples to determine the arterial concentration of total radioactivity in plasma and whole blood as well as to analyse arterial plasma concentration of metabolites. This requires the cannulation of the radial artery, with principally similar risks as described for venous sampling. Subjects will receive a local anaesthetic around the anticipated arterial puncture site, using e.g. 1-2 mL of 1% lidocaine. Although the risk associated to the s.c. injection of a local anaesthetic is considered low, rare occurrence of hypersensitivity reactions have been reported. Since the radial artery is not an 'end' artery the ulnar artery can completely supply the hand in isolation. The radial artery cannulation is considered a safe procedure. However, temporary occlusion, isolated cases of permanent ischaemic damage or the development of a pseudoaneurysm have been described. For mitigation of such risks the arterial puncture will be only conducted by highly skilled medical staff, confirmation of adequate collateral circulation with the Allen's test, and close monitoring of the injection site.

There is minimal risk to subjects associated with MRI scanning, provided subjects have no contraindications to MRI as listed in the exclusion criteria. Some participants may find the PET and MRI scanning environments claustrophobic. Participants with severe anxiety of confined spaces will be excluded from the study. The potential risks associated with metallic implants will be eliminated by a careful screening of the subject during study enrolment procedure and by a qualified MR operator on the day of the scan.

Drug-related risks and safety measures

The human safety and tolerability profile in male and female healthy subjects was satisfactory for multiple doses given over 28 consecutive days of up to 20 mg of BI 1467335 in study 1386.8 (see Section 1.2). There were no deaths or other serious adverse events. Adverse events, mostly reported as mild (CTCAE grade 1), had no apparent dose or exposure relationship. In view of the non-proportional increase of exposure there is an about 10-fold safety margin between the highest exposure observed in preceding phase I studies and the exposure at the maximum dose of 10 mg of BI 1467335 q.d. in the planned study.

Drug-induced liver injury (DILI)

Although rare, a potential for drug-induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety; see also Section 5.2.6.1.4, adverse events of special interest.

Summary of benefit-risk assessment

In a previous trial in healthy subjects, multiple oral doses of up to 20 mg BI 1467335 were safe and well-tolerated. Due to the non-proportional increase of the exposure this results in a large safety margin between the exposure at the already tested maximum doses and the maximum dose of 10 mg of BI 1467335 in the planned study. Each participating subject will receive on 3 occasions 14 days apart a single intravenous bolus of [^{11}C]-L-deprenyl-D2' at a low dose which is not expected to have a noteworthy pharmacological effect. Furthermore, the low dose should result in a negligible radioburden per infusion (ICRP 1 category).

The trial design is optimized to collect as much relevant information as possible on the inhibition of MAO-B in the brain upon treatment with BI 1467335 without exposing participating volunteers to undue risk. However, there is always the potential of serious adverse events (SAEs) occurring with intake of trial medication. Risks to subjects will be minimized and addressed by eligibility criteria, safety laboratory examinations, ECG and vital sign measurements, in-house observation periods, and verbal communication concerning AEs.

If the investigator should have any clinical concern, the safety of the subjects will be of paramount importance. The investigator has the discretion to remove subjects from the study should there be any safety concerns or if the subject's wellbeing is at jeopardy.

The results of this trial are considered necessary for the further development of BI 1467335. Successful development of BI 1467335 is expected to provide a new valuable treatment for patients with NASH and potentially also for non-proliferative diabetic retinopathy.

The risks of the participating volunteers are minimized and justified when compared to the potential benefits of this trial.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main objective of this trial is to investigate the effect of multiple oral dosing of 10 mg BI 1467335 over 28 days and multiple oral dosing of 3 mg BI 1467335 over 42 days on MAO-B occupancy in the brain compared to baseline using [^{11}C]-L-deprenyl-D2 PET tracer in healthy male subjects.

2.1.2 Primary endpoint

The following primary endpoint will be determined:

- % reduction in MAO-B availability upon treatment with BI 1467335 on the last treatment day (Day 28 for the 10 mg dose group and Day 42 for the 3 mg dose group) compared to baseline.

2.1.3 Secondary endpoints

The following secondary endpoints will be determined:

- % reduction in MAO-B availability upon treatment with 10 mg BI 1467335 on Day 14 compared to baseline.
- % reduction in MAO-B availability upon treatment with 3 mg BI 1467335 on Day 28 compared to baseline.
- MAO-B inhibition in platelet rich plasma at Day 14 (10 mg dose group only), Day 28, and Day 42 (3 mg dose group only) compared to baseline.
- $C_{max,N}$ (maximum measured concentration of the analyte) and $AUC_{0-24,\,N}$ (area under the concentration-time curve of the analyte over the time interval from 0 to 24 h) on Day 14 (10 mg dose group only), Day 28 and Day 42 (3 mg dose group only).

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

The study will be performed as a non-randomised, open-label, parallel-group, multiple dose trial in healthy male subjects in order to investigate the effect of multiple oral doses of 10 mg BI 1467335 administered once daily over 28 days and of 3 mg BI 1467335 administered once daily over 42 days on MAO-B activity in the brain. The MAO-B activity will be assessed by means of a dynamic PET scan using [^{11}C]-L-deprenyl-D2 PET as tracer. PET scans will be conducted predose and on Day 28 for both dose groups. An additional PET scan will be conducted on Day 14 in the 10 mg dose group and on Day 42 in the 3 mg dose group. On these days, subjects will be injected with the radiotracer and subsequently undergo dynamic PET scans. In addition to PET scan, MAO-B activity will be also measured in platelet rich plasma.

For the 10 mg dose group, subjects will be hospitalised from Day -1 to Day 29.

For the 3 mg dose group, subject will be hospitalised from Day -1 to Day 43. The subjects will be allowed to leave the clinical unit in the afternoon on Day 7 and Day 14.

The 10 mg dose group will be investigated first. In case no MAO-B inhibition is seen in this dose group, the 3 mg dose group will not be further investigated.

The oral treatments will consist of either two 5 mg or three 1 mg film-coated tablets (dose strength based on free base) for subjects dosed with 10 mg and 3 mg BI 1467335, respectively. The treatments will be administered once daily for 28 days for the 10 mg dose groups and once daily for 42 days for the 3 mg dose groups. The subjects willing to participate can choose convenient visit dates on a first-come, first-served basis: as soon as 5 subjects have been allocated to 1 of the 2 dose groups, the following subjects will be allocated to the other dose group. For details, refer to Section 4.1.

An overview of all relevant trial activities is provided in the Flow Chart. For visit schedule and details of trial procedures at selected visits, refer to Sections 6.1 and 6.2, respectively.

3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP

The doses selected for this study, 10 mg BI 1467335 q.d. (2 film-coated tablets of 5 mg dose strength) and 3 mg BI 1467335 q.d. (3 film-coated tablets of 1 mg dose strength), have been used in healthy volunteers and shown to be well tolerated. The predose measurement will be used as a baseline, so no placebo dose is required in this study.

The planned dosing duration of 28 days for the 10 mg dose group and 42 days for the 3 mg dose group is long enough to reliably achieve steady-state drug exposures and to exclude any transient effects.

Due to the time dependent non-linear kinetics of BI 1467335, systemic exposures on Day 28 and Day 42 cannot be predicted from Day 1 kinetics (IB [c04751792]). Therefore the MAO-B inhibition in the brain will be determined on Day 28 and Day 42 after start of treatment

with 10 mg and 3 mg BI 1467335, respectively, and compared to the individual baseline value to investigate the maximum inhibition. The *in vitro* data suggested an irreversible inhibition, which could potentially accumulate over time following multiple dosing and with the increase in exposure observed over time which characterizes BI 1467335 kinetics. The investigation on Day 14 for the 10 mg dose group and on Day 28 for the 3 mg dose group will evaluate whether the MAO-B inhibition reaches its maximum over the treatment duration and is maintained regardless of the increase in BI 1467335 systemic exposure. The 10 mg dose group will be investigated first. In the event no MAO-B inhibition is seen following the administration of 10 mg BI 1467335 daily over 28 days, the study will be considered as complete and no further investigation with a lower dose (3 mg) will be conducted.

[^{11}C]-L-deprenyl-D2 is a PET radioligand used for measurement of the MAO-B activity in the brain. This radioligand has been used for several PET studies to evaluate MAO-B inhibition in the brain in healthy subjects and is unlikely to exhibit any detectable isotope effects (see Section 1.4).

Because of the exposure with radioligands the study will only include male subjects.

The open-label treatment is not expected to bias results, since the study endpoints are derived from medical procedures and laboratory assessments with an unlikely relevant impact by subjective factors.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 10 healthy male subjects (5 in each dose group) will enter the study. They will be recruited from the volunteers' pool of the trial site.

The current trial is designed to investigate the effect of multiple oral dosing of 10 mg BI 1467335 over 28 days and 3 mg BI 1467335 over 42 days on MAO-B inhibition in the brain. Healthy male subjects are an ideal population for the objectives of this trial, since they provide relatively stable physiological, biochemical and hormonal conditions, i.e. the absence of disease-related variations and relevant concomitant medications.

A log of all subjects enrolled into the trial (i.e. who have signed informed consent) will be maintained in the ISF irrespective of whether they have been treated with investigational drug or not.

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

Subjects will only be included in the trial if they meet the following criteria:

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 21 to 55 years (inclusive)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive)
4. Signed and dated written informed consent prior to admission to the study, in accordance with GCP and local legislation
5. Non-smoker with no history of smoking
6. Subjects who are sexually active must use, with their partner, highly effective contraception from the time of administration of trial medication until 4 months after administration of trial medication. Adequate methods are:
   - Condoms *plus* use of hormonal contraception by the female partner that started at least 2 months prior to administration of trial medication (e.g., implants, injectables, combined oral or vaginal contraceptives, intrauterine device) *or*
   - Condoms plus surgical sterilization (vasectomy at least 1 year prior to enrolment) or
   - Condoms plus surgically sterilised partner (including hysterectomy) or
   - Condoms plus intrauterine device or
   - Condoms plus partner of non-childbearing potential (including homosexual men)

Subjects are required to use condoms to prevent unintended exposure of the partner to the study drug via seminal fluid.

Alternatively, true abstinence is acceptable when it is in line with the subject's preferred and usual lifestyle. If a subject is usually not sexually active but becomes active, with their partner, they must comply with the contraceptive requirements detailed above.

3.3.3 Exclusion criteria

Subjects will not be allowed to participate, if any of the following general criteria apply:

1. Any finding in the medical examination (including BP, PR or ECG) deviating from normal and assessed as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders

8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 90 days or within 5 half-lives, whichever is longer, of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered
13. Alcohol abuse (consumption of more than 30 g per day for males) within the 24 months prior to dosing
14. Drug abuse within the 24 months prior to dosing or positive drug screening
15. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
16. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
17. Inability to comply with the dietary regimen of the trial site
18. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening
19. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
20. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study

In addition, the following trial-specific exclusion criteria apply:

21. Structural brain abnormality has been shown on an MRI
22. Severe anxiety of enclosed spaces
23. Contraindication for arterial cannulation: Allen's test indicating potential risk in placement of the arterial cannula.
24. Contraindication to MRI as determined by screening and safety questionnaire including but not limited to significant tattoos (as determined by the radiographer), cardiac pacemakers, aneurysm clips and cochlear implants.
25. Unable to lie flat on the MRI or PET scanner for a prolonged period of time.
26. Prior participation in other research protocols in the past year such that the total effective dose including this study would exceed 10 mSv.

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

Subjects may discontinue trial treatment or withdraw consent to trial participation as a whole ('withdrawal of consent') with very different implications; please see Sections 3.3.4.1 and 3.3.4.2 below.

If a subject is removed from or withdraws from the trial prior to the first administration of trial medication, the data of this subject will not be entered in the case report form (CRF) and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after the first administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF; in addition, the data will be included in the CRF and will be reported in the CTR. At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRF. If the discontinuation occurs before the end of the REP (see Section 1.2.2), the discontinued subject should if possible be questioned for AEs and concomitant therapies at or after the end of the REP in order to ensure collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

1. The subject wants to discontinue trial treatment, without the need to justify the decision
2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future.
3. The subject needs to take concomitant medication that interferes with the investigational medicinal product or other trial treatment
4. The subject can no longer receive trial treatment for medical reasons (such as surgery, adverse events [AEs], or diseases)
5. The subject has an elevation of AST and/or ALT ≥3-fold ULN and an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the DILI checklist provided in the ISF
6. In addition to these criteria, the investigator may discontinue subjects at any time based on his or her clinical judgment.

Even if the trial treatment is discontinued, the subject remains in the trial and, given his/her agreement, will undergo the procedures for early treatment discontinuation and follow up as outlined in the Flow Chart and Section 6.2.3.

3.3.4.2 Withdrawal of consent to trial participation

Subjects may withdraw their consent to trial participation at any time without the need to justify the decision. If a subject wants to withdraw consent, the investigator should be involved in the discussion with the subject and explain the difference between trial treatment

discontinuation and withdrawal of consent to trial participation, as well as explain the options for continued follow up after trial treatment discontinuation, please see Section 3.3.4.1 above

3.3.4.3 Discontinuation of the trial by the sponsor

Boehringer Ingelheim reserves the right to discontinue the trial at any time for any of the following reasons:

1. Failure to meet expected enrolment goals overall or at a particular trial site
2. New toxicological findings, serious adverse events, or any safety information invalidating the earlier positive benefit-risk-assessment. More specifically, the trial will be terminated if more than 50% of the subjects have drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least 1 drug-related serious adverse event is reported
3. Violation of GCP, or the CTP, or the contract with BI impairing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the investigational product
5. The investigator / trial site will be reimbursed for reasonable expenses incurred in case of trial termination (except if item 3 applies).
6. Severe non-serious adverse reactions (i.e. severe non-serious adverse events considered as, at least, possibly related to the IMP administration) in two subjects in the same dose group, independent of within or not within the same system-organ-class.

3.3.5 **Replacement of subjects**

In case a subject does not complete the trial, the Clinical Trial Leader together with the Trial Pharmacokineticist and the Trial Statistician are to decide, if this subject will be replaced. Replacement of subjects should always be done in mutual agreement with the principal investigator. A replacement subject will be assigned a unique trial subject number, and will be assigned to the same treatment as the subject replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

The investigational product has been manufactured by BI Pharma GmbH & Co. KG. BI 1467335 doses will be calculated as a free base of the BI 1467335 salt, with 10 mg BI 1467335 free base equals to 11.3 mg BI 1467335 HCl salt and 3 mg BI 1467335 free base equals to 3.39 mg BI 1467335 HCl salt.

[^{11}C]-L-deprenyl-D2 will be administered as a solution for injection and will be manufactured by

4.1.1 Identity of the Investigational Medicinal Product

The characteristics of the test products are given below:

Substance:	BI 1467335
Pharmaceutical formulation:	Film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	5 mg /1 mg
Posology:	2-0-0 / 3-0-0
Route of administration:	oral
Duration of use:	28 days / 42 days

4.1.2 Identity of the Non Investigational Medicinal Product

The characteristics of the NIMP are given below:

Substance:	[^{11}C]-L-deprenyl-D2
Pharmaceutical formulation:	Solution for injection
Source:	
Unit strength:	≤100 µg
Route of administration:	i.v.
Duration of use:	A single dose on 3 different days during study conduct

4.1.3 Selection of doses in the trial

The doses of BI 1467335 selected for this trial are the standard clinical doses (see Section 1.2).

4.1.4 Method of assigning subjects to treatment groups

This trial is an open-label, non-randomized, parallel-group, multiple dose study.

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate can choose convenient visit dates on a first-come, first-served basis: as soon as 5 subjects have been allocated to 1 of the 2 dose groups, the following subjects will be allocated to the other dose group. Therefore, the allocation of subjects to dose groups is not influenced by trial personnel, but only by the subjects' temporal availability. As the study includes healthy male subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

Within the dose group, numbers of the randomization list will be allocated to subjects in their order of registration for the study (assignment to dose group according to subjects' temporal availability). Once a subject number has been assigned, it cannot be reassigned to any other subject.

4.1.5 Drug assignment and administration of doses for each subject

This trial is a parallel-group multiple dose study. All subjects will receive the treatments in an open-labelled fashion. The treatments to be evaluated are outlined in Table 4.1.5: 1 below.

Table 4.1.5: 1 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage
T (Test)	BI 1467335	Film-coated tablet	10 mg	2 tablets (5 mg) qd for 28 days
T (Test)	BI 1467335	Film-coated tablet	3 mg	3 tablets (1 mg) qd for 42 days

The investigator (or authorised designee) will administer the trial medication as an oral dose together with about 240 mL of water to subjects who are in a sitting position. For drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, one authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation (e.g. reconstitution), if correct dosage cannot be ensured otherwise.

[^{11}C]-L-Deprenyl-D2 will be administered as an intravenous bolus injection for a predose assessment (between Day -14 to Day -2) and about 1,5 h after dosing of BI 1467335 on Day 14 (10 mg dose group only), Day 28 and Day 42 (3 mg dose group only).

4.1.6 Blinding and procedures for unblinding

This Phase I trial will be handled in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations.

Emergency envelopes will not be provided, because the dose of trial medication is known to investigators and subjects.

4.1.7 Packaging, labelling, and re-supply

The investigational medicinal products will be provided by BI. They will be packaged and labelled in accordance with local law and the principles of Good Manufacturing Practice.

The PET tracer will be manufactured by

For details of packing and the description of the label, refer to the ISF.

The telephone number of the sponsor and the name, address and telephone number of the trial site are provided in the subject information form. The EudraCT number is indicated on the title page of this protocol as well as on the subject information and informed consent forms.

No re-supply is planned.

4.1.8 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area in accordance with the recommended (labelled) storage conditions. If necessary, a temperature log must be maintained to make certain that the drug supplies are stored at the correct temperature. If the storage conditions are found to be outside the specified range, the local clinical trial manager (as provided in the list of contacts) is to be contacted immediately.

4.1.9 Drug accountability

The investigator or designee will receive the IMPs delivered from the sponsor provided that following requirements are fulfilled:

- Approval of the clinical trial protocol by the IRB / ethics committee
- Availability of a signed and dated clinical trial contract between the sponsor and the investigational site
- Approval/notification of the regulatory authority, e.g. competent authority
- Availability of the *curriculum vitae* of the Principal Investigator
- Availability of a signed and dated clinical trial protocol

Only authorised personnel documented in the form 'Trial Staff List' may dispense medication to trial subjects. The trial medication must be administered in the manner specified in the CTP.

The investigator or designee must maintain records of the product’s delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products. These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational medicinal product and trial subjects. The investigator or designee will maintain records that document adequately that the subjects were provided the doses specified by the CTP and reconcile all investigational medicinal products received from the sponsor. At the time of disposal of remaining trial medication, the investigator or designee must verify that all unused or partially used drug supplies have been returned by the clinical trial subject and that no remaining supplies are in the investigator’s possession.

All unused medication will be disposed of locally by the trial site upon written authorisation of the clinical trial leader. Receipt, usage and disposal of trial medication must be documented on the appropriate forms. Account must be given for any discrepancies.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed. No additional treatment is planned. However, if adverse events require treatment, the investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed. All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF. Limited amounts of paracetamol (up to 2 g/day) are allowed when prescribed by a medical investigator from the clinical site.

4.2.2.2 Restrictions on diet and life style

While admitted to the trial site, the subjects will be instructed not to consume any foods or drinks other than those provided by the staff. Standardised meals will be served at the times indicated in the Flow Chart.

Grapefruits, Seville oranges (sour or bitter oranges) and their juices, and dietary supplements and products containing St. John's wort (*Hypericum perforatum*) are not permitted from 7 days before the first administration of trial medication until after the last PK sample of each dose group is collected.

Alcoholic beverages are not allowed 48 h before administration of the compound, during the conduct of the study and up discharge from clinic, as well as 48 h before End of Trial.

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed during the in-house period.

Poppy-seed containing products should not be consumed starting 2 days before first trial drug administration until last PK sampling of the trial.

Excessive physical activity (such as competitive sport) should be avoided from 7 days before the first administration of trial medication until the end of trial examination.

4.3 TREATMENT COMPLIANCE

Compliance will be assured by administration of all trial medication in the study centre under supervision of the investigating physician or a designee. The measured plasma concentrations of trial medication will provide additional confirmation of compliance.

Subjects who are non-compliant (for instance, who do not appear for scheduled visits or violate trial restrictions) may be removed from the trial and the CRF will be completed accordingly (for further procedures, please see Section 3.3.4.1).

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

Not applicable.

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

At screening, the medical examination will include demographics, height and body weight, smoking history (non-smoker mandatory) and alcohol history (results not mandatory to be entered into CRF or to be reported), relevant medical history and concomitant therapy, review of inclusion and exclusion criteria, review of vital signs (BP, PR), 12-lead ECG, laboratory tests, and a physical examination (including Allen's test to check arterial blood flow of the hands). At discharge and at the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination including determination of weight.

5.2.2 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) or heart rate (heart rate is considered to be equal to pulse rate) will be measured by a blood pressure monitor (e.g. Dinamap Pro 1000) at the times indicated in the Flow Chart, after subjects have rested for at least 5 min in a supine position. All recordings should be made using the same type of blood pressure recording instrument on the same arm, if possible.

5.2.3 Safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the times indicated in the Flow Chart after the subjects have fasted for at least 10 h. For retests, at the discretion of the investigator or designee, overnight fasting is not required.

The parameters that will be determined are listed in Tables 5.2.3: 1 and 5.2.3: 2. Reference ranges will be provided in the ISF, Section 10.

Manual differential white blood cell count and urine light microscopy will only be performed if there is an abnormality in the automatic blood cell count or in the urinalysis, respectively. The results of the manual differential white blood cell and urine light microscopy will only be reported in the source data.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A	B	C	D	E
Haematology	Haematocrit	X	X	X	X	X
	Haemoglobin	X	X	X	X	X
	Red Blood Cell Count/Erythrocytes	X	X	X	X	X
	Reticulocytes, absol.	X	X	X	X	X
	White Blood Cells/Leucocytes	X	X	X	X	X
	Platelet Count/Thrombocytes (quant)	X	X	X	X	X
Automatic WBC differential, relative	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes	X	X	X	X	X
Automatic WBC differential, absolute	Neutrophil, absol.; Eosinophils, absol.; Basophils, absol.; Monocytes, absol.; Lymphocytes, absol.	X	X	X	X	X
Manual differential WBC (if automatic differential WBC is abnormal)	Eosinophils/Leukocytes; Eosinophils, absol.; Basophils/ Leukocytes; Basophils, absol.; Monocytes/ Leukocytes; Monocytes, absol.; Lymphocytes/Leukocytes; Lymphocytes, absol.					
Coagulation	Activated Partial Thromboplastin Time	X	X	X	X	X
	Prothrombin time – INR (International Normalization Ratio)	X	X	X	X	X
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X	X	X
	Alkaline Phosphatase	X	X	X	X	X
	Gamma-Glutamyl Transferase	X	X	X	X	X
	Creatine Kinase [CK]	X	X	X	X	X
	Creatine Kinase Isoenzyme MB [only if CK is elevated]	X	X	X	X	X
	Lactic Dehydrogenase (LDH)	X	X	X	X	X
	Lipase	X				X
	Amylase (total)	X				X
Hormones	Thyroid Stimulating Hormone	X				X
Substrates	Glucose (Plasma)	X	X	X	X	X
	Creatinine	X	X	X	X	X
	Bilirubin, Total	X	X	X	X	X
	Bilirubin, Direct	X	X	X	X	X
	Protein, Total	X	X	X	X	X
	Albumin	X	X	X	X	X
	Alpha glycoprotein acid	X	X	X	X	X
	C-Reactive Protein (Quant)	X				X
	Uric Acid	X	X	X	X	X
	Urea	X	X	X	X	X
	Cholesterol, total	X	X	X	X	X
	Triglyceride	X	X	X	X	X

Table 5.2.3: 1 Routine laboratory tests (cont.)

Functional lab group	BI test name [comment/abbreviation]	A	B	C	D	E
Electrolytes	Sodium	X	X	X	X	X
	Potassium	X	X	X	X	X
	Magnesium	X	X	X	X	X
	Calcium	X	X	X	X	X
Urinalysis (Stix)	Urine Nitrite (qual)	X	X	X	X	X
	Urine Protein (qual)	X	X	X	X	X
	Urine Glucose (qual)	X	X	X	X	X
	Urine Ketone (qual)	X	X	X	X	X
	Urobilinogen (qual)	X	X	X	X	X
	Urine Bilirubin (qual)	X	X	X	X	X
	Urine RBC/Erythrocytes (qual)	X	X	X	X	X
	Urine WBC/Leucocytes (qual)	X	X	X	X	X
	Urine pH	X	X	X	X	X
Urine light microscopy (microscopic examination if erythrocytes, leukocytes nitrite or protein are abnormal in urine)	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)					

A: parameters to be determined at Visit 1 (screening examination)
B: parameters to be determined at Visit 2 on Day 1 (for time points refer to Flow Chart)
C: parameters to be determined at Visit 2 on Day 14 (for time points refer to Flow Chart)
D: parameters to be determined at Visit 2 on Day 28 (for time points refer to Flow Chart)
E: parameters to be determined at Visit 3 (end of trial examination)

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Except for drug screening, it is planned to perform these tests during screening only.

Table 5.2.3: 2 Exclusionary laboratory tests

Functional lab group	Test name
Drug screening (urine)	Amphetamine Barbiturates Benzodiazepine Cannabis Cocaine Methadone Methamphetamines Opiates Phencyclidine Tricyclic antidepressants
Infectious serology (blood)	Hepatitis B surface antigen (qualitative) Hepatitis B core antibody (qualitative) Hepatitis C antibodies (qualitative) HIV-1 and HIV-2 antibody (qualitative)

To encourage compliance with alcoholic restrictions, a urine alcohol test (nal von minden DrugScreen®) will be performed prior to first administration, and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR. Drug screening also includes a cotinine test to confirm compliance with non-smoking.

The laboratory tests listed in Tables 5.2.3: 1 and 5.2.3: 2 will be performed at Safety Laboratory –
with the exception of drug screening tests and urinalysis. These tests will be performed at the trial site using nal von minden DrugScreen® kits and Siemens Multistix® or comparable test system.

Laboratory data will be transmitted electronically to the database.

5.2.4 Electrocardiogram

Twelve-lead ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph at the times provided in the Flow Chart.

To achieve a stable heart rate at rest and to assure high quality recordings, the site personnel will be instructed to assure a relaxed and quiet environment, so that all subjects are at complete rest.

All ECGs will be recorded for a 10-sec duration after subjects have rested for at least 5 min in a supine position. ECG assessment will always precede all other study procedures scheduled for the same time to avoid compromising ECG quality.

All ECGs will be stored electronically. Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven.

All locally printed ECGs will be evaluated by the investigator or a designee. Abnormal findings will be reported as AEs (during the trial) or baseline conditions (at screening) if assessed to be clinically relevant by the investigator. Any ECG abnormalities will be

carefully monitored and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

ECGs may be repeated for quality reasons (for instance, due to alternating current artefacts, muscle movements, or electrode dislocation) and the repeated ECG will be used for analysis. Additional (unscheduled) ECGs may be collected by the investigator for safety reasons.

5.2.5 Other safety parameters

Not Applicable.

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of adverse events

5.2.6.1.1 Adverse event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

The following should also be recorded as an AE in the CRF and BI SAE form (if applicable):

- Worsening of the underlying disease or of other pre-existing conditions
- Changes in vital signs, ECG, physical examination, and laboratory test results, if they are judged clinically relevant by the investigator

If such abnormalities already pre-exist prior to trial inclusion, they will be considered as baseline conditions and should be collected in the eCRF only.

5.2.6.1.2 Serious adverse event

A serious adverse event (SAE) is defined as any AE which fulfils at least one of the following criteria:

- Results in death
- Is life-threatening, which refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe
- Requires inpatient hospitalisation
- Requires prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Is deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgment which may jeopardise the patient and may

require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse

5.2.6.1.3 AEs considered 'Always Serious'

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the trial medication and must be reported as described in Section 5.2.6.2, subsections 'AE Collection' and 'AE reporting to sponsor and timelines'.

In accordance with the European Medicines Agency initiative on Important Medical Events, Boehringer Ingelheim has set up a list of further AEs, which, by their nature, can always be considered to be 'serious' even though they may not have met the criteria of an SAE as defined above.

The latest list of 'Always Serious AEs' can be found in the eDC system, an electronic data capture system which allows the entry of trial data at the trial site.

These events should always be reported as SAEs as described above.

5.2.6.1.4 Adverse events of special interest

The term adverse events of special interest (AESI) relates to any specific AE that has been identified at the project level as being of particular concern for prospective safety monitoring and safety assessment within this trial, e.g. the potential for AEs based on knowledge from other compounds in the same class. AESIs need to be reported to the sponsor's Pharmacovigilance Department within the same timeframe that applies to SAEs, please see Section 5.2.6.2.2.

The following are considered as AESIs:

- Hepatic injury
  A hepatic injury is defined by the following alterations of hepatic laboratory parameters:
  - o An elevation of AST (aspartate transaminase) and/or ALT (alanine transaminase) ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN measured in the same blood sample, or
  - o Aminotransferase (ALT, and/or AST) elevations ≥10 fold ULN

  These lab findings constitute a hepatic injury alert and the subjects showing these lab abnormalities need to be followed up according to the 'DILI checklist' provided in the eDC system. In case of clinical symptoms of hepatic injury (icterus, unexplained encephalopathy, unexplained coagulopathy, right upper quadrant abdominal pain, etc.) without lab results (ALT, AST, total bilirubin) available, the Investigator should make sure that these parameters are analysed, if necessary in an unscheduled blood

test. Should the results meet the criteria of hepatic injury alert, the procedures described in the DILI checklist should be followed.

5.2.6.1.5 Intensity (severity) of AEs

The intensity (severity) of the AE should be judged based on the following:

Mild: Awareness of sign(s) or symptom(s) that is/are easily tolerated
Moderate: Sufficient discomfort to cause interference with usual activity
Severe: Incapacitating or causing inability to work or to perform usual activities

5.2.6.1.6 Causal relationship of AEs

Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

Arguments that may suggest that there is a reasonable possibility of a causal relationship could be:

- The event is consistent with the known pharmacology of the drug
- The event is known to be caused by or attributed to the drug class
- A plausible time to onset of the event relative to the time of drug exposure
- Evidence that the event is reproducible when the drug is re-introduced
- No medically sound alternative aetiologies that could explain the event (e.g. pre-existing or concomitant diseases, or co-medications)
- The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g. Stevens-Johnson syndrome)
- An indication of dose-response (i.e. greater effect size if the dose is increased, smaller effect size if dose is reduced)

Arguments that may suggest that there is no reasonable possibility of a causal relationship could be:

- No plausible time to onset of the event relative to the time of drug exposure is evident (e.g. pre-treatment cases, diagnosis of cancer or chronic disease within days / weeks of drug administration; an allergic reaction weeks after discontinuation of the drug concerned)
- Continuation of the event despite the withdrawal of the medication, taking into account the pharmacological properties of the compound (e.g. after 5 half-lives). Of note, this criterion may not be applicable to events whose time course is prolonged despite removing the original trigger
- Additional arguments amongst those stated before, like alternative explanation (e.g. situations where other drugs or underlying diseases appear to provide a more likely explanation for the observed event than the drug concerned)

- Disappearance of the event even though the trial drug treatment continues or remains unchanged

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 AE collection

Upon enrolment into a trial, the subject's baseline condition is assessed (for instance, by documentation of medical history/concomitant diagnoses), and relevant changes from baseline are noted subsequently.

Subjects will be required to report spontaneously any AEs as well as the time of onset, end time, and intensity of these events. In addition, each subject will be regularly assessed by the medical staff throughout the clinical trial and whenever the investigator deems necessary. As a minimum, subjects will be questioned for AEs (and concomitant therapies) at the time points indicated in the Flow Chart. Assessment will be made using non-specific questions such as 'How do you feel?'. Specific questions will be asked wherever necessary in order to more precisely describe an AE.

A carefully written record of all AEs shall be kept by the investigator in charge of the trial. Records of AEs shall include data on the time of onset, end time, intensity of the event, and any treatment or action required for the event and its outcome.

The following must be collected and documented on the appropriate CRF(s) by the investigator:

- From signing the informed consent onwards until an individual subject's end of trial:
    - All AEs (serious and non-serious) and all AESIs
    - The only exception to this rule are AEs (serious and non-serious) and AESIs in Phase I trials in healthy volunteers, when subjects discontinue from the trial due to screening failures prior to administration of any trial medication. In these cases, the subjects' data must be collected at trial site but will not be entered in the CRF or trial database and will not be reported in the CTR.
- After the individual subject's end of trial:
    - The investigator does not need to actively monitor the subject for AEs but should only report any occurrence of cancer and related SAEs and related AESIs of which the investigator may become aware of by any means of communication, e.g. phone call. Those AEs should, however, not be reported in the CRF.

5.2.6.2.2 AE reporting to the sponsor and timelines

The Investigator must report SAEs, AESIs, and non-serious AEs which are relevant for the reported SAE or AESI, on the BI SAE form via fax immediately (within 24 hours) to the sponsor's unique entry point (country specific contact details will be provided in the ISF). The same timeline applies if follow-up information becomes available. In specific occasions the Investigator could inform the sponsor upfront via telephone. This does not replace the requirement to complete and fax the BI SAE form.

With receipt of any further information to these events, a follow-up SAE form has to be provided. For follow-up information, the same rules and timeline apply as for initial information.

5.2.6.2.3 Information required

All (S)AEs, including those persisting after the individual subject's end of trial, must be followed up until they have resolved, have been sufficiently assessed as 'chronic' or 'stable', or no further information can be obtained.

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

For the assessment of pharmacokinetics, blood samples will be collected at the time points indicated in the Flow Chart. The actual sampling times will be recorded and used for determination of pharmacokinetic parameters. The sampling scheme is to be adhered to as closely as possible, but on the days of the PET scans, any PK samples that would need to be taken during the PET scan procedures, may be taken earlier or later, if needed.

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis

For quantification of BI 1467335, concentrations in plasma, approx. 3 mL of blood will be drawn from a vein into an K_2-EDTA (dipotassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venepuncture with a metal needle.

For detailed description of blood sampling, sample handling, sample preparation, sample storage, tube labelling, and sample shipment refer to the laboratory manual. Until transfer on dry ice to the analytical laboratory, the plasma samples will be stored frozen and in upright position at about -20°C or below at the clinical site and at the analytical laboratory until analysis.

At a minimum, the sample tube labels should list BI trial number, subject number, visit, and planned time point, analyte, and aliquot.

After completion of the trial, the plasma samples may be used for further methodological investigations (e.g. for stability testing or assessment of metabolites). However, only data related to the analyte and/or its metabolite(s) including anti-drug antibodies (if applicable) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the CTR is archived.

5.3.3 Analytical determinations

5.3.3.1 Analytical determination of BI 1467335, plasma concentrations

BI 1467335, concentrations in plasma will be determined by a validated LC-MS/MS (liquid chromatography tandem mass spectrometry) assay.

All details of the analytical method will be available prior to the start of sample analysis.

5.4 ASSESSMENT OF BIOMARKER

5.4.1 Platelet-enriched plasma

5.4.1.1 Assessment

For the assessment of platelet-enriched plasma, blood samples will be collected at the time points indicated in the Flow Chart. The actual sampling times will be recorded.

5.4.1.2 Method of sample collection

Blood will be withdrawn into citrate monovettes. After collection, blood was centrifuged at 200 g for 10 min at RT. Supernatants (platelet rich plasma) will be aliquoted and stored at -20 °C ±10 °C. Details regarding the collection of the platelet-enriched plasma will be specified in a dedicated laboratory manual.

5.5 MRI AND PET PROCEDURE

5.5.1 MRI and PET Scan

Each subject will report to the PET site on 4 occasions:

- Screening visit: A screening brain MRI will be performed and interpreted at the PET site to determine eligibility of the subjects by ruling out any brain abnormality. Subject's brain MR images will be evaluated by a radiologist to exclude incidental findings of clinical relevance. In the unlikely event of an unexpected and clinical significant finding on structural MRI, which may require further investigation,

participants and/or their General Practitioner will be informed, with the participant's consent. In the case of a subject being re-screened, an MRI (without any abnormality) obtained at the study's imaging center made within 90 days prior to Day -1 check-in is acceptable for eligibility.

- Day -14 to Day -2: Subjects will be admitted to the PET site after confirmation of eligibility at the clinical site. They will be administered [^{11}C]-L-deprenyl-D2 as PET ligand through an i.v. bolus and will undergo dynamic PET scans from 0 to 90 min following the administration of the PET ligand.

- Day 14 (10 mg dose group only)/Day 28/Day 42 (3 mg dose group only): Subjects will be admitted to the PET site in the morning, with intake of the daily drug administration at the PET site (to assure start of PET scans 90 minutes after dosing of BI 1467335). They will receive an intravenous bolus injection of the tracer ([^{11}C]-L-deprenyl-D2) and will then undergo dynamic PET scans from 0 to 90 min. After completion of the PET scan subjects will be re-admitted to the clinical trial site.

5.5.2 Arterial blood sampling

Arterial blood will be collected continuously for 15 min using an automated blood sampling system at a speed of 5 mL/min. Arterial blood will also be drawn manually after the ligand injection to measure radioactivity in whole blood and plasma. Actual sampling times will be detailed in an Arterial Blood Sampling manual, a possible schedule could be 1, 2, 4, 6, 8, 10, 15, 20, 30, 45, and 60 min after ligand injection.

The parent fraction of the ligand will be determined using a reversed-phase HPLC method. Sample handling instructions will be included in the arterial blood sampling protocol.

5.6 BIOBANKING

Not applicable.

5.8 APPROPRIATENESS OF MEASUREMENTS

Measurements performed during this trial are mainly standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The PET scan is an established in-vivo tool to evaluate potential MAO B occupancy by drugs, with a large body of evidence regarding the validity of the method. The assessment of MAO-B activity in platelet-rich plasma will support the results of the PET scan thus further substantiating potential signals on MAO B occupancy.

The safety assessments performed during the study are widely used in clinical trials and will allow evaluation of safety and tolerability of the study drugs.

The pharmacokinetic parameters and measurements outlined in Section 5.4 are generally used assessments of drug exposure.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

Exact times of measurements outside the permitted time windows will be documented. The acceptable time windows for screening and the end of trial examination are provided in the Flow Chart.

Study measurements and assessments scheduled to occur 'before' trial medication administration on Day 1 are to be performed and completed within a 3 h-period prior to the trial drug administration.

The acceptable deviation from the scheduled time for study drug administration will be ±1 h, however dosing time within a subject should be the same.

For PET scans and PK blood sampling planned times, refer to the Flow Chart. The acceptable deviation from the scheduled days for PET scans and associated PK blood sampling will be 1 day before up to 2 days after planned study day on Day 14, Day 28 and Day 42. On PET days, the acceptable deviation from the scheduled time for PET scans and associated PK blood sampling will be ± 2 h. While the nominal times for PK blood sampling should be adhered to as closely as possible, the actual sampling times will be recorded and used for the determination of pharmacokinetic parameters.

If scheduled in the Flow Chart at the same time as a meal, blood sampling, vital signs, and 12-lead ECG recordings have to be done first. Furthermore, if several measurements including venepuncture are scheduled for the same time, venepuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters.

If a subject misses an appointment, it will be rescheduled if possible. The relevance of measurements outside the permitted time windows will be assessed no later than at the Report Planning Meeting.

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

Screening visit is defined as Visit 1.

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study.

For information regarding laboratory tests (including drug and virus screening), ECG, vital signs, and physical examination, refer to Sections 5.2.3 to 5.2.5.

6.2.2 Treatment period

Each subject is expected to participate in 1 treatment period.

Each study participants will receive a once daily administration of BI 1467335 of either 10 mg (2 x 5 mg film-coated tablets) from Day 1 to Day 28 or 3 mg (1 x 3 mg film-coated tablets) from Day 1 to Day 42.

For the 10 mg dose group, the subjects will be kept at the clinical site from Day -1 up to Day 29, at which time they will be allowed to leave the trial site after formal assessment and confirmation of their fitness.

For the 3 mg dose group, the subjects will be kept at the clinic from Day -1 to Day 43. However on Day 7 and Day 14, subjects will be allowed to leave the clinic in the afternoon at the discretion of the Investigator. Before subjects are allowed to leave the trial site on Day 43, they will undergo formal assessments and confirmation of their fitness.

Between Day -14 and Day -2 and on Day 14 (for the 10 mg dose group only), Day 28 and Day 42 (for the 3 mg dose group only), each study participants will be admitted to the PET site for a PET ligand administration followed by a PET scan. After completion of PET scan on Day 14, 28 and Day 42, subjects will be re-admitted to the clinical trial site.

For details on time points and procedures for collection of plasma samples for PK analysis and platelet-enriched plasma analysis, refer to Flow Chart and Section 5.3.2.

The safety measurements performed during the treatment period are specified in Section 5.3 of this protocol and in the Flow Chart. For details on times of all other trial procedures, refer to the Flow Chart. AEs and concomitant therapy will be assessed continuously from screening until the end of trial examination.

6.2.3 Follow-up period and trial completion

For AE assessment, laboratory tests, recording of ECG and vital signs, and physical examination during the follow-up period, see Sections 5.2.2 to 5.2.5.
Subjects who discontinue treatment before the end of the planned treatment period should undergo the EoTrial Visit.

All abnormal values (including laboratory parameters) that are assessed as clinically relevant by the investigator will be monitored using the appropriate tests until a return to a medically acceptable level is achieved. (S)AEs persisting after a subject's EoTrial Visit must be followed until they have resolved, have been sufficiently characterised, or no further information can be obtained.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

The main objective of this trial is to investigate the effect of multiple oral dosing of 10 mg BI 1467335 over 28 days and 3 mg BI 1467335 over 42 days on MAO-B occupancy in the brain compared to baseline using [^{11}C]-L-deprenyl-D2 PET tracer on the basis of the primary and secondary endpoints, as listed in Section 2.1.2 and 2.1.3.

A second objective of this trial is to investigate the MAO-B inhibition in platelet-enriched plasma compared to baseline.

The assessment of safety and tolerability is a further objective of this trial, and will be evaluated by descriptive statistics for the parameters specified in Section 2.2.2.2.

7.2 NULL AND ALTERNATIVE HYPOTHESES

It is not planned to test any statistical hypothesis with regard to the primary and secondary endpoints in a confirmatory sense. Instead, they will be described in their entirety and evaluated by descriptive statistical methods.

7.3 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets:

- Treated set (TS): The treated set includes all subjects who were randomized and treated with at least one dose of study drug. The treated set will be used for safety analyses.
- Pharmacokinetic parameter analysis set (PKS): This set includes all subjects in the treated set (TS) who provide at least one PK endpoint and was not excluded due to a protocol deviation relevant to the evaluation of PK or due to PK non-evaluability (as specified in the following subsection ‘Pharmacokinetics’). Thus, a subject will be included in the PKS, even if he/she contributes only one PK parameter value to the statistical assessment. Analyses of PK parameters will be based on the PKS.
- PET set (PETS): This set includes all subjects in the treated set (TS) who provide at least one PET scan at baseline and one on-treatment.

Adherence to the protocol will be assessed by the trial team. Important protocol deviations (iPD) categories will be suggested in the integrated risk management plan (IQRMP), iPDs will be identified no later than in the Report Planning Meeting, and the iPD categories will be updated as needed.

Pharmacokinetics

The pharmacokinetic parameters listed in Section 2.1 and 2.2 for BI 1467335, will be calculated according to the relevant SOP of the Sponsor [001-MCS-36-472].

Plasma concentration data and parameters of a subject will be included in the statistical pharmacokinetic (PK) analyses if they are not flagged for exclusion due to a protocol deviation relevant to the evaluation of PK (to be decided no later than in the Report Planning Meeting) or due to PK non-evaluability (as revealed during data analysis, based on the criteria specified below). Exclusion of a subject's data will be documented in the CTR.

Relevant protocol deviations may be

- Incorrect trial medication taken, i.e. the subject received at least one dose of trial medication the subject was not assigned to
- Incorrect dose of trial medication taken
- Use of restricted medications

Plasma concentrations and/or parameters of a subject will be considered as non-evaluable, if for example

- The subject experienced emesis that occurred at or before two times median t_{max} of the respective treatment (median t_{max} is to be determined excluding the subjects experiencing emesis),
- Missing samples/concentration data at important phases of PK disposition curve

Plasma concentration data and parameters of a subject which is flagged for exclusion will be reported with its individual values but will not be included in the statistical analyses. Descriptive statistics of PK parameters will be based on the PKS.

Only concentration values within the validated concentration range and actual sampling times will be used for the calculation of pharmacokinetic parameters. Concentrations used in the pharmacokinetic calculations will be in the same format provided in the bioanalytical report, (that is, to the same number of decimal places provided in the bioanalytical report).

Biomarkers

All scans for which good quality PET emission and arterial plasma radioligand concentration data will be obtained, will be included in the analysis.

7.3.1 Primary endpoint analyses

Image analysis will be used to generate the outcome parameter for the PET data (proportional to the target availability at each PET scan), using the PET emission and the metabolite corrected arterial plasma input function, within an appropriate kinetic model. Tissue time-activity curves (TACs) for each region of interest (ROI) will be analysed together with arterial blood data, processed to generate metabolite-corrected arterial plasma input function curves. Kinetic modelling techniques will then be applied to the input function and TAC data to estimate parameters relating to the level of target availability for each PET scan. For scans

following 14, 28 or 42 days of dosing with BI 1467335, fractional occupancy values will be generated for each post-dose PET scan by comparing the outcome parameters for baseline and post-dose scans for a subject (primary endpoint). In addition to the individual values, descriptive statistics per dose group will be provided.

The occupancy data will be related to the plasma concentration of BI 1467335 at the time of the PET scan.

7.3.2 Secondary endpoint analyses

Secondary endpoints:

- % reduction in MAO-B availability upon treatment with 10 mg BI 1467335 on Day 14 compared to baseline.
- % reduction in MAO-B availability upon treatment with 3 mg BI 1467335 on Day 28 compared to baseline.
- MAO-B inhibition in platelet rich plasma at Day 14 (10 mg dose group only), Day 28 and Day 42 (3 mg dose group only) compared to baseline.

Will be analysed similarly to the primary endpoints described in Section 7.3.1 above.

The secondary endpoints C_{max} and AUC_{0-24} of BI 1467335 will be only evaluated descriptively. The following descriptive statistics will be calculated: N, arithmetic mean, standard deviation, minimum, median, maximum, arithmetic coefficient of variation, geometric mean, and geometric coefficient of variation. The data format for descriptive statistics of concentrations will be identical with the data format of the respective concentrations. The descriptive statistics of PK parameters will be calculated using the individual values with the number of decimal places as provided by the evaluation program. Then the individual values as well as the descriptive statistics will be reported with 3 significant digits in the clinical trial report.

7.3.4 Safety analyses

Safety will be analysed based on the assessments described in Section 2.2.2.2. All treated subjects (TS, refer to Section 7.2) will be included in the safety analysis. Safety analyses will be descriptive in nature and based on BI standards. No hypothesis testing is planned.

For all analyses, the treatment actually administered (= treatment at onset) to the subject will be used (any deviations from the randomised treatment will be discussed in the minutes of the Report Planning Meeting).

Treatments will be compared in a descriptive way. Tabulations of frequencies/proportions will be used to evaluate categorical (qualitative) data, and tabulations of descriptive statistics will be used to analyse continuous (quantitative) data.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to the screening period, those between first trial medication intake and end of REP (which corresponds to the patient's individual end of treatment; see Section 1.2.2) will be assigned to the treatment period. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Note that AEs occurring after the last per protocol contact but entered before final database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

Additionally, further treatment intervals (analysing treatments) may be defined in the TSAP in order to provide summary statistics for time intervals, such as combined treatments, on-treatment totals, or periods without treatment effects (such as screening and follow-up intervals).

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity, and causal relationship of AEs will be tabulated by treatment, system organ class, and preferred term. SAEs, AESIs (see Section 5.2.6.1), and other significant AEs (according to ICH E3) will be listed separately.

Previous and concomitant therapies will be presented per treatment group without consideration of time intervals and treatment periods.

Laboratory data will be compared to their reference ranges. Values outside the reference range as well as values defined as possibly clinically significant will be highlighted in the listings. Additionally, differences from baseline will be evaluated.

Vital signs or other safety-relevant data will be assessed with regard to possible on-treatment changes from baseline.

Relevant ECG findings will be reported as AEs.

7.4 INTERIM ANALYSES

No formal interim analysis is planned, however a preliminary analysis of MAO-B inhibition after completion of the 10 mg dose group will be performed.

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

It is not planned to impute missing values for safety parameters.

7.5.2 Pharmacokinetics

Handling of missing PK data will be performed according to the relevant Corporate Procedure [001-MCS-36-472].

PK parameters that cannot be reasonably calculated based on the available drug concentration-time data will not be imputed.

7.6 RANDOMISATION

Randomisation is not applicable in this open-label, parallel group study. A list of consecutive random numbers for each treatment arm (10 mg and 3 mg) will be provided (see Section 4.1.3 for details of allocation of random numbers to subjects).

7.7 DETERMINATION OF SAMPLE SIZE

For this exploratory study, no prospective calculations of statistical precision or power have been made. The planned sample size of 5 subjects per dose group has been selected for practical reasons and is judged as being adequate to get reliable results regarding the trial objectives.

8. INFORMED CONSENT, TRIAL RECORDS, DATA PROTECTION, PUBLICATION POLICY, AND ADMINISTRATIVE STRUCTURE

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for Good Clinical Practice (GCP), relevant BI Standard Operating Procedures (SOPs), the EU regulation 536/2014, and other relevant regulations. Investigators and site staff must adhere to these principles.

Standard medical care (prophylactic, diagnostic, and therapeutic procedures) remains the responsibility of the subject's treating physician.

The investigator will inform the sponsor immediately of any urgent safety measures taken to protect the trial subjects against any immediate hazard, as well as of any serious breaches of the protocol or of ICH GCP.

The Boehringer Ingelheim transparency and publication policy can be found on the following web page: trials.boehringer-ingelheim.com. The rights of the investigator and of the sponsor with regard to publication of the results of this trial are described in the investigator contract. As a general rule, no trial results should be published prior to archiving of the CTR.

The terms and conditions of the insurance coverage are made available to the investigator and the subjects, and are stored in the ISF.

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB) / Independent Ethics Committee (IEC), the Administration of Radioactive Substances Advisory Council (ARSAC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.
Prior to a subject's participation in the trial, written informed consent must be obtained from each subject (or the subject's legally accepted representative) according to ICH-GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional subject-information form retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional subject information must be given to each subject or the subject's legally accepted representative.

The subject must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the subject's own free will with the informed consent form after confirming that the subject understands the contents. The investigator or delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.

Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor's instructions.

The consent and re-consenting process should be properly documented in the source documentation.

8.2 DATA QUALITY ASSURANCE

A risk-based approach is used for trial quality management. It is initiated by the assessment of critical data and processes for trial subject protection and reliability of the results as well as identification and assessment of associated risks. An Integrated Quality and Risk Management Plan documents the rationale and strategies for risk management during trial conduct including monitoring approaches, vendor management and other processes focusing on areas of greatest risk.

Continuous risk review and assessment may lead to adjustments in trial conduct, trial design or monitoring approaches.

A quality assurance audit/inspection of this trial may be conducted by the sponsor, sponsor's designees, or by IRB / IEC or by regulatory authorities. The quality assurance auditor will have access to all medical records, the investigator's trial-related files and correspondence, and the informed consent documentation of this clinical trial.

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

In accordance with regulatory requirements, the investigator should prepare and maintain adequate and accurate source documents and trial records for each trial subject that include all observations and other data pertinent to the investigation. Source data as well as reported data should follow the 'ALCOA principles' and be attributable, legible, contemporaneous, original, and accurate. Changes to the data should be traceable (audit trail).

Data reported on the CRF must be consistent with the source data or the discrepancies must be explained.

Before providing any copy of subjects' source documents to the sponsor, the investigator must ensure that all subject identifiers (e.g., subject's name, initials, address, phone number, and social security number) have properly been removed or redacted to ensure subject confidentiality.

If the subject is not compliant with the protocol, any corrective action (e.g. re-training) must be documented in the subject file.

For the CRF, data must be derived from source documents, for example:

- Subject identification: sex, year of birth (in accordance with local laws and regulations)
- Subject participation in the trial (substance, trial number, subject number, date subject was informed)
- Dates of subject's visits, including dispensing of trial medication

- Medical history (including trial indication and concomitant diseases, if applicable)
- Medication history
- AEs and outcome events (onset date [mandatory], and end date [if available])
- SAEs (onset date [mandatory], and end date [if available])
- Concomitant therapy (start date, changes)
- Originals or copies of laboratory results and other imaging or testing results, with proper documented medical evaluation (in validated electronic format, if available)
- ECG results (original or copies of printouts)
- Completion of subject's participation in the trial (end date; in case of premature discontinuation, document the reason for it, if known)
- Prior to allocation of a subject to a treatment into a clinical trial, there must be documented evidence in the source data (e.g. medical records) that the trial participant meets all inclusion criteria and does not meet any exclusion criteria. The absence of records (either medical records, verbal documented feedback of the subject or testing conducted specific for a protocol) to support inclusion/exclusion criteria does not make the subject eligible for the clinical trial.

8.3.2 Direct access to source data and documents

The investigator /institution will allow site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access must be provided to the CRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must be available at all times for review by the Clinical Research Associate, auditor and regulatory inspector (e.g. FDA). They may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in Section 8.3.1. The sponsor will also monitor compliance with the protocol and GCP.

8.3.3 Storage period of records

Trial site:
The trial site must retain the source and essential documents (including ISF) according to contract or the local requirements valid at the time of the end of the trial (whatever is longer).

Sponsor:
The sponsor must retain the essential documents according to the sponsor's SOPs.

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

BI is responsible to fulfil their legal and regulatory reporting obligation in accordance with regulatory requirements.

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

Individual subject data obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the exceptions noted in Section 8.7.

Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the principles 6 and 12 of the WHO GCP handbook.

Personalised treatment data may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated at the site as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB / IEC and the regulatory authorities.

8.5.1 Collection, storage and future use of biological samples and corresponding data

Measures are in place to comply with the applicable rules for the collection and future use of biological samples and clinical data, in particular

- Sample and data usage has to be in accordance with the informed consent
- The BI-internal facilities storing biological samples from clinical trial participants are qualified for the storage of biological samples collected in clinical trials.
- An appropriate sample and data management system, incl. audit trail for clinical data and samples to identify and destroy such samples according to ICF is in place
- A fit for the purpose documentation (biomarker proposal, analysis plan and report) ensures compliant usage

Samples and/or data may be transferred to third parties and other countries as specified in the ICF.

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date of the enrolment of the first subject in the trial.

The **end of the trial** is defined as the 'date of the last visit of the last subject in whole trial' ('Last Subject Completed') or 'end date of the last open AE' or 'date of the last follow-up test' or 'date of an AE has been decided as sufficiently followed-up', whichever is latest.

Early termination of the trial is defined as the premature termination of the trial for any reason before the end of the trial as specified in this protocol.

Temporary halt of the trial is defined as any unplanned interruption of the trial by the sponsor with the intention to resume it.

Suspension of the trial is defined as an interruption of the trial based on a Health Authority request.

The EC/competent authority in each participating EU member state will be notified about the trial milestones according to the laws of each member state.

A final report of the clinical trial data will be written only after all subjects have completed the trial in all countries (EU or non-EU), so that all data can be incorporated and considered in the report.

The sponsor will submit to the EU database a summary of the final trial results within one year from the end of a clinical trial as a whole, regardless of the country of the last patient (EU or non-EU).

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

The trial will be conducted

under the supervision of the Principal Investigator. Relevant documentation on the participating (Principal) Investigators (e.g. their curricula vitae) will be filed in the ISF.

The PET procedure will be conducted at

under the supervision of an Investigator.

BI has appointed a Clinical Trial Leader, responsible for coordinating all required trial activities, in order to

- Manage the trial in accordance with applicable regulations and internal SOPs
- Direct the clinical trial team in the preparation, conduct, and reporting of the trial
- ensure appropriate training and information of local clinical trial manager (CTM), Clinical Research Associates, and investigators of participating trial sites

The trial medication will be provided by the

Safety laboratory tests will be performed by the local laboratory of the trial site

Analyses of BI 1467335 concentrations in plasma will be performed at
. Analysis of the platelet-enriched plasma will be performed at

On-site monitoring will be performed by BI or a contract research organisation appointed by BI.

Data management and statistical evaluation will be done by BI according to BI SOPs.

Tasks and functions assigned in order to organise, manage, and evaluate the trial are defined according to BI SOPs. A list of responsible persons and relevant local information can be found in the ISF.

9. REFERENCES

9.1 PUBLISHED REFERENCES

P13-02280 Torres DM, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2012; 10:837-58.

R15-5365 Weston CJ, Shepherd EL, Claridge LC et al. Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. Journal of Clinical Investigation. 2015; 125:501-520.

R15-5697 Karim S, Liaskou E, Fear J, Garg A, Reynolds G, Claridge L, Adams DH, Newsome PN, Lalor PF Dysregulated hepatic expression of glucose transporters in chronic disease: contribution of semicarbazide-sensitive amine oxidase to hepatic glucose uptake. Am J Physiol 307 (12), G1180 - G1190 (2014)

R15-6044 Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association Hepatology. 2012; 55:2005-2023.

R15-6057 Murphy SL, Xu J, Kochanek KD. Deaths: final data for 2010. Natl Vital Stat Rep 2013;61(4):1-117.

R15-6070 Dharel N, Fuchs M. Nonalcoholic Fatty Liver Disease – A Major Public Health Challenge for the 21st Century. JSM Gastroenterology and Hepatology. 2014; 1018.

R18-2789 Logan J, Fowler JS, Volkow ND, Wang G-J, MacGregor RR, Shea C. Reproducibility of repeated measures of deuterium substituted [11C]L-deprenyl ([11C]L-deprenyl-D2) binding in the human brain. Nucl. Med. Biol. 2000; 27:43-49.

R18-3939 Fowler JS, Logan J, Volkow ND, Wang G-J. Translational neuroimaging: positron emission tomography studies of monoamine oxidase. Mol. Imaging Biol. 2005; 7: 377-387.

R18-3940 Lammertsma AA, Bench CJ, Price GW, Cremer JE, Luthra SK, Turton D, Wood ND, Frackowiak RSJ. Measurement of Cerebral Monoamine Oxidase B Activity Using L-[11C]Deprenyl and Dynamic Positron Emission Tomography. J. Cereb. Blood Flow Metab. 1991; 11: 545-556.

R18-3941 Fowler JS, Logan J, Wang G-J, Volkow ND, Telang F, Ding Y-S, Shea C, Garza V, Xu Y, Li Z, Alexoff D, Vaska P, Ferrieri R, Schlyer D, Zhu W, Gatley SJ. Comparison of the binding of the irreversible monoamine oxidase tracers, [11C]clorgyline and [11C]l-deprenyl in brain and peripheral organs in humans. Nucl. Med. Biol. 2004; 31: 313-319.

R18-3942 Fowler JS, Volkow ND, Wang GJ, Logan J, Pappas N, Shea C, MacGregor R. Age-related increases in brain monoamine oxidase B in living healthy human subjects. Neurobiol Aging 1997; 18: 431-435.

R18-3943 Kumlien E, Nilsson A, Hagberg G, Långström B, Bergström M. PET with 11C-deuterium-deprenyl and 18F-FDG in focal epilepsy. Acta Neurol. Scand 2001; 103: 360-366.

R18-3950 Fowler JS, Volkow ND, Cilento R, Wang G-J, Felder C, Logan J. Comparison of brain glucose metabolism and monoamine oxidase B (MAO B) in traumatic brain injury. Clin. Pos. Imag. 1999; 2:71-79.

R18-3964 Cephalon (UK) Limited. Zelapar - Summary of Product Characteristics (SmPC). 28 September 2015

9.2 UNPUBLISHED REFERENCES

001-MCS-36-472 Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics. Current version

c04751792 . Investigator's Brochure: BI 1467335 in NASH and NPDR. 29 May 2018.

c08854973 Safety, tolerability, pharmacokinetics and pharmacodynamics of multiple rising oral doses of BI 1467335 in healthy male and female subjects (double-blind, randomised, placebo-controlled within dose groups). 1386.8. 05 Oct 2016.

c08980589 A multi-centre, double-blind, parallel-group, randomised, placebo controlled phase II a study to investigate safety, tolerability, pharmacodynamics, and pharmacokinetics of different doses of orally administered BI 1467335 during a 12-week treatment period compared to placebo in patients with clinical evidence of NASH. 1386.4. 12 Sep 2018.

c09036683 Single ascending dose and multiple ascending dose phase 1 study of PXS-4728A administered orally in healthy adult males. PXS-4728A-101. 13 Nov 2015.

c14141887 . A Randomized, double-masked, placebo-controlled exploratory study to evaluate safety, tolerability, pharmacodynamics and pharmacokinetics of Orally administered BI 1467335 for 12 weeks with a 12 week follow up period in patients with Non-proliferative diabetic retinopathy without center-involved diabetic macular edema (ROBIN study). 1386.12. 01 Aug 2018.

c16567028 . 8-07-other-preliminary-analysis_10+15mg-appendix2. 1386.8 . 28 Mar 2017.

c17150058 Relative bioavailability of a BI 1467335 tablet compared to a BI 1467335 oral solution and the effect of food on the bioavailability of the tablet following oral adminsitartion (a randomised, open-label, single dose, three-way crossover trial in helathy male subjects). 1386.17. 09 Nov 2017.

n00244592 BI 1467335: 4-week oral (gavage) supplementary toxicity study in Sprague Dawley rats. 15B108. 06 Jul 2016

n00247850 . A Four-Week Oral Repeat Dose Toxicity and Toxicokinetic Study of PXS-4728A in Beagle Dogs. 53703-13-825. 04 Nov 2014.

n00252396 . Excretion in urine, faeces and bile after oral and intravenous administration of [14C]BI 1467335 to rats. A096/15SMB. 18 Oct 2016.

n00252404 . Pharmacokinetics of radioactivity and BI 1467335 after oral and intravenous administration of [14C]BI 1467335 to rats. B5778. 18 Oct 2016.

n00257993 In vitro inhibition of MAO-A and MAO-B catalyzed kynuramine deamination by BI 1467335. A437/17LU. 31 Aug 2017.

n00259383 Irreversible in vitro inhibition of MAO-B catalyzed kynuramine deamination by BI 1467335. A442/17LU. 01 December 2017.

10. APPENDICES

Not applicable.

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		24 June 2019
EudraCT number **EU number**		2018-003745-41
BI Trial number		1386-0022
BI Investigational Medicinal Product		BI 1467335
Title of protocol		A Phase I, open-label, Positron Emission Tomography study in healthy male subjects to explore the inhibition of monoamine oxidase B in the brain after multiple oral doses of BI 1467335 (non-randomized, open-label, parallel-group study)

To be implemented only after approval of the IRB / IEC / Competent Authorities	☒
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval	☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only	☐

Section to be changed		1. Flow Chart: 3 mg Dose Group 2. Flow Chart: 10 Dose Group 3. Section 1.3: Rationale for performing the trial 4. Section 3.1: Overall trial design and plan 5. Section 3.2: Discussion of trial design, including the choice of control group 6. Section 5.3.4: Pharmacokinetic-pharmacodynamic relationship 7. Section 6.1: Visit Schedule 8. Section 8.7: Administrative structure of the trial
Description of change		1. Flow Chart 3 mg Dose Group: Urine drugs, alcohol breath test and urine nicotine have been amended to improve compliance by performing these tests upon readmission to the site on Day 7 and 14 when the subjects are allowed to leave the clinic. In addition, a minor clarification regarding footnote 2 has been added, which states that predose PK sampling after Day 1 should be collected within 30 min before the following dose.

		2. Flow Chart 10 mg Dose Group: A minor discrepancy regarding the planned PK time points and EOT time window was corrected. In addition, a minor clarification regarding footnote 2 has been added, which states that predose PK sampling after Day 1 should be collected within 30 min before the following dose. 3. Section 1.3: Rationale for performing the trial An additional decision point was included in the trial: the 10 mg dose group will be investigated first and in case no inhibition of MAO-B is seen in this group, further investigation of the 3 mg dose group will not be performed and the study will be considered as complete. 4. Section 3.2: Discussion of trial design, including the choice of control group Description of the additional decision point as outlined in bullet point 3. 5. Section 3.2: Discussion of trial design, including the choice of control group Description of the additional decision point as outlined in bullet point 3. 6. Section 5.3.4: Pharmacodynamic-pharmacodynamic relationship Description of this section was omitted in the previous version, the information that no formal analysis of the relationship is planned was added. 7. Section 6.1 Visit schedule Clarification regarding deviation window allowance for ECGs, Vital signs and Safety laboratory has been added. 8. Section 8.7: Administrative structure of the trial Laboratories for PK analysis and biomarker analysis have been corrected.
Rationale for change		Following discussion with the Ethics committee, an additional decision point was included in the trial in order to ensure that the minimum number of subjects

		are investigated. In addition, correction of discrepancies and minor changes in administrative structure were amended in the context of the amendment.

11.2 GLOBAL AMENDMENT 2

Date of amendment		
EudraCT number **EU number**		
BI Trial number		
BI Investigational Medicinal Product(s)		
Title of protocol		
To be implemented only after approval of the IRB / IEC / Competent Authorities		☐
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☐
Section to be changed		
Description of change		
Rationale for change		

APPROVAL / SIGNATURE PAGE

Document Number: c25939630 **Technical Version Number:2.0**

Document Name: clinical-trial-protocol-version-02

Title: A Phase I, open-label, Positron Emission Tomography study in healthy male subjects to explore the inhibition of monoamine oxidase B in the brain after multiple oral doses of BI 1467335 (non-randomized, open-label, parallel-group study)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Clinical Trial Leader		24 Jun 2019 11:34 CEST
Approval-Therapeutic Area		24 Jun 2019 13:00 CEST
Approval-Team Member Medicine		24 Jun 2019 13:35 CEST
Author-Trial Statistician		25 Jun 2019 11:01 CEST
Author-Trial Clinical Pharmacokineticist		26 Jun 2019 15:19 CEST
Verification-Paper Signature Completion		27 Jun 2019 06:35 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed